A Bromine Compound Existing in Blood

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YANAGISAWA, I. and TORII, S. *A Bromine Compound Existing in Blood.* Tohoku J. Exp. Med., 2002, 196(3), 111-121 — Since a bromine compound with REM-sleep-inducing and anti-choline esterase activities have been isolated from human cerebrospinal fluid, and was identified as 1-methylheptyl γ -bromoacetoacetate, the compound was chemically synthesized. It was found that this compound was composed with three forms, i.e., a keto-form, an enol-form that changed gradually from keto-form by tautomerism, and a stable six-membered ring form (=cyclic γ -Br) converted from enol form, when it was chemically synthesized. In addition, it was found that the six-membered ring form of this bromine compound was present in the human blood. However, in this case, the keto-form and the enol-form were not detected. When ¹⁴C-butyrate was injected to rats, it was incorporated into the bromine compound in the blood of the animal and the bromine compound formed was found to be present mainly as the six-membered ring form. From these results, the mechanism for the formation of bromine compounds in human and animal blood were deduced. ——— bromine compound; 1-methylheptyl γ -bromoacetoacetate; cyclic γ -Br
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Yanagisawa and Yoshikawa (1967, 1968) firstly reported that there exists higher concentration of bromine in human cerebrospinal fluid (CSF), and that most of the bromine was involved in an organic compound. The organic bromine compound later isolated by Yanagisawa and Yoshikawa (1973) from human CSF was identified as 1-methylheptyl γ -bromoacetoacetate.

On the other hand, the short chain fatty acids such as n-butyrate and γ -hydroxybutyrate were reported to exert REM-sleep inducing action in cats, but the doses

necessary for inducing REM-sleep was as high as 0.5 to 1.0 m mol/kg (Juvet et al. 1961; Matsuzaki et al. 1964; Winters et al. 1965; Matsuzaki and Takagi 1967). Later, Torii and Yanagisawa (1973), showed that REM-sleep was induced, when 0.0003 m mol/kg of 1-methylheptyl γ -bromoacetoacetate was administered to the encephalo isolate cat. It was indicated that the REM-sleep would be caused by anti-choline esterase action of this compound (Yamane et al. 1984). Therefore, 1-methylheptyl γ -bromoacetoacetate seems to be a possible candidate for inducing REM-sleep in

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animals and humans.

After the report of Yanagisawa and Yoshikawa (1973) on 1-methylheptyl γ -bromoacetoacetate an endogeneous human CSF constituent as stated above, it has been suggested by a number of studies that a humoral factor inducing sleep might exist in the blood and the CSF. Prostaglandin D₂ as a slow wave sleep (SWS) inducer (Ueno et al. 1982), adenosine as an endogenous regulator of sleep (Porkka-Heiskanen et al. 1997), and other compounds such as cholecystokinin, endotoxin, growth hormone, interleukin-1, muramyl peptides, and tumor necrosis factor (Inoue 1989) have been demonstrated as substances related with sleep induction. However, many of these molecules are not found in the brain or CSF. Though 1-methylheptyl γ -bromoacetoacetate, a bromine compound exists in CSF, this compound seems not to be universally accepted as a single causative agent of sleep, and it has been unclear how this compound exerts its effects at the molecular level. Recently, Boger et al. (1998) demonstrated that oleamide is an endogenous sleep-inducing lipid to modulate serotonergic neurotransmission and inhibit intercellular gap junction communication, and that fatty acid amide hydrolase that degrades oleamide is potentially inhibited by 1-methylheptyl γ -bromoacetoacetate. This fact may elucidate the action of 1-methylheptyl γ -bromoacetoacetate on the induction of sleep at the molecular level.

Thus, it seems to be of significance, at present, to study the distribution of 1-methylheptyl γ -bromoacetoacetate in the body, especially, in the blood, because the levels of this compound in the blood have not been investigated hitherto. The author decided to study whether 1-methylheptyl γ -

bromoacetoacetate may exist in the human blood. In the present manuscript, the existence of 1-methylheptyl γ -bromoacetoacetate in the human blood was studied, and the form of the compound in the blood was analyzed in detail, because the author found that the chemically synthesized 1-methylheptyl γ -bromoacetoacetate is composed of three forms, keto-form, enol-form and cyclic form. Furthermore, we studied the incorporation of n-butyrate into cyclic form of 1-methylheptyl γ -bromoacetoacetate after the administration of ¹⁴C-butyrate to rats, because n-butyrate has been considered as a possible precursor of 1-methylheptyl γ -bromoacetate in the body.

MATERIALS AND METHODS

Materials

Bromine was purchased from Tokyo Kasei Co., Ltd. (Tokyo). DANCYL L-cysteine was freshly prepared from Di-DANSYL L-cystine (Sigma Chemical Co., St. Louis, MO, USA) according to the method of Wilson et al. (1974), and kept on ice. Thin layer chromatography (TLC) plates, 20×20 cm, and 5×20 cm were prepared with a 0.5 mm layer of silica gel containing 13% CaSO₄ (Wako gel B-10, Wako Pure Chemicals, Tokyo) and activated for 24 hours at 110°C. NaB-³H₄ and sodium ¹⁴C-butyrate were purchased from Amersham Laboratories Co., Ltd. (Little Chalfont, Buckinghamshire, UK) Filter paper (No. 5A, Toyo Roshi, Tokyo) was washed well with ethanol to eliminate any fluorescent substances and stored in ethanol.

Human blood plasma were obtained by centrifuging the heparinized blood, which was freshly obtained after the informed consents from a healthy adult, at 3000 rpm for 20 minutes.

DANSYL L-cysteine, 5 (dimethylamino)-1-naphthalene sulfonyl L-cysteine; NMR, nuclear magnetic resonance; REM sleep, rapid eye movement sleep; ³H- γ -Br, 1-³H-1-methylheptyl γ -bromoacetoacetate; γ -Br, 1-methylheptyl γ -bromoacetoacetate; keto γ -Br, keto form of 1-methylheptyl γ -bromoacetoacetate; enol γ -Br, enol form of 1-methylheptyl γ -bromoacetoacetate; cyclic γ -Br, cyclic form of 1-methylheptyl γ -bromoacetoacetate; DOC, di-1-methylheptyl 2, 5-dioxycyclo-hexane 1, 4-dicarboxylate.

Synthesis of ^3H - γ -Br

Synthesis of ^3H - γ -Br was performed as follows. Eight mg NaBH_4 was mixed with 5 mCi $\text{NaB-}^3\text{H}_4$ dissolved in 0.25 ml of 0.1 N NaOH (specific activity: 465 mCi/mg, Amersham Laboratories Co., Ltd.) and 49 mg 2-octanone (0.4 m mol) was added. One ml of 70% ethylamine solution was added to form a turbid mixture which was clarified by the addition of several drops of water. The mixture was allowed to stand for 48 hours at room temperature and acidified to about pH 1.0 with 2 N HCl . A few ml of the acidified solution was extracted several times with one ml aliquots of CCl_4 . The CCl_4 extracts were combined and dried over anhydrous sodium sulfate overnight. $1\text{-}^3\text{H}$ -1-methylheptanol was contained in the extract. In an ice bath, 64 mg of Br_2 (0.4 m mol) dissolved in 5 ml CCl_4 was added dropwise to 33.5 mg of diketene (about 0.4 m mol) dissolved in 5 ml of CCl_4 . Under cooling in an ice bath, the $1\text{-}^3\text{H}$ -1-methylheptanol solution was added dropwise to the brominated diketene solution. The reaction mixture was washed several times with a few ml of water and the mixture was dried overnight over anhydrous sodium sulfate. The solvent was evaporated in vacuo and the oily residue was re-dissolved with a small amount of chloroform. The solution was applied to a silicic column of 7 mm $\times$ 100 mm and developed by chloroform. Fractions of the eluate showing positive reaction to 0.1% FeCl_3 methanol solution were collected and the solvent was evaporated off in vacuo. The residue was 68.2 mg for a calculated yield of 60%.

Nonlabeled 1-methylheptyl γ -bromoacetoacetate (γ -Br) was synthesized by the method described previously (Yanagisawa and Yoshikawa 1973). Thin layer chromatography of the ^3H -labeled and the nonlabeled γ -Br was carried out on thin layer plates developed by a solvent mixed with CCl_4 and CHCl_3 (1:1 v/v).

Thin layer chromatography of bromine compounds labeled with DANSYL L-cysteine was carried out at 5°C, using Solvent I (chloroform: hexane: acetonitrile: benzene: acetone: dioxane: acetic acid = 30:30:15:10:10:5:1, v/v) for the primary development and Solvent III (acetone: hexane: acetic acid = 400:160:1, v/v) for the secondary development. Fluorescent spots were detected under ultraviolet light.

Radioactivity measurement and NMR spectrum measurement

Radioactivity was measured using a scintillation spectrometer 0.1 to 1.0 ml samples were mixed with 10 ml Scientisol EX-H (Wako Pure Chemicals, Tokyo), and were used for measurement of radioactivity. The NMR spectrum was measured by an R-90H type NMR apparatus (Hitachi, Tokyo).

Measurement of fluorescence of DANSYL L-cysteine derivatives of γ -Br

Fluorescence intensity of the DANSYL L-cysteine derivatives of γ -Br was measured at an excitation wave length of 360 nm and an emission wave length of 520 nm against a standard solution of DANSYL glycine (Wako Pure Chemicals). A blank experiment was performed, because some material with a fluorescence emission spectrum at about 420 nm was present in the TLC plates. Namely, an area of TLC gel of the similar size and close to the spots was extracted for the blank examination.

The qualitative fluorescence measurement was made at a low temperature using a Shimadzu RF530 fluorescence spectrometer (Shimadzu, Kyoto), because the spectra were unstable at warmer temperature.

Detection of enol-form of 1-methylheptyl γ -bromoacetoacetate

Detection of enol-form of 1-methylheptyl γ -bromoacetoacetate was carried out according to the method of Meyer (1918). Briefly, spots of DANSYL L-cysteine derivatives of 1-

methylheptyl γ -bromoacetoacetate on a TLC plate were extracted with a known amount of cold ethanol. Then, ethanol was evaporated in a test tube under reduced pressure. Saturated bromine water was added dropwise to the remnant to obtain a yellow color. The solution was then decolorized by the addition of sulfurous acid, excess of which was removed by boiling. After cooling, one drop each of 5% KI solution and 1% starch solution were added to the test tube. The presence of an enol was detected by the appearance of a purple color in the iodine-starch reaction.

Labeling of ^3H - γ -Br with DANSYL L-cysteine

A few ml of methanol solution containing a few n mol of ^3H - γ -Br which consists of cyclic γ -Br and keto γ -Br in the ratio of 30 : 70, one ml of DANSYL L-cysteine solution and 5 ml of 14% ethylamine solution (about pH 11.0) were mixed. The mixture was allowed to stand at room temperature for thirty minutes and then condensed to about one ml in vacuo. The condensed solution was acidified to about pH 1 with 2 N HCl and extracted four times with a few ml of ethylether. These extracts were combined, washed with about one ml of water and dried over anhydrous sodium sulfate. The ether was evaporated to dryness in a conical centrifuge tube. The residue was dissolved in a few drops of ethanol and applied to TLC. The TLC was developed primary by the solvent I and secondary by the solvent III.

Treatment of human blood plasma with DANCYL L-cysteine and detection of the bromine compounds in the plasma

Five ml of heparinized plasma was acidified to pH 1.0 with 2 N HCl, and mixed with 150 ml of a solvent mixed with chilled ethanol and ether (3:1). The mixture was filtered into a vessel through a filter paper. One ml of the DANSYL L-cysteine solution and 20 ml of 14% ethylamine were added to the filtrate. The mixture was condensed to a few

ml by evaporation in vacuo at a temperature below 10°C. Most of ethylamine was eliminated by the evaporation. Finally, one ml of the DANSYL L-cysteine and one ml of 14% ethylamine were added to the condensed turbid solution and the mixture was left to stand for 10 minutes at room temperature. The mixture was then adjusted to about pH 1.0 by addition of 2 N HCl and the acidified mixture was extracted several times with a few ml of ethyl ether. The ether extracts were combined and washed once with 1 ml of water. Then the extract was dried over anhydrous sodium sulfate. An aliquot of the ether extract was condensed to residue by evaporation and dissolved in a small amount of ethanol with undissolved matter remaining. The ethanol solution was applied to TLC and developed two dimensionally. These proceedings have to be carried under lower temperature as far as possible.

Administration of $1\text{-}^{14}\text{C}$ -butyrate into rats

Sodium $1\text{-}^{14}\text{C}$ -butyrate (4 mCi) mixed with 20 mg of nonlabeled sodium butyrate dissolved in 5 ml of physiological saline and portions containing 0.2 mCi to 0.5 mCi of the solution were intraperitoneally injected to male albino rats. After 30 minutes, rats were anesthetized by sodium phenobarbital (Wako Pure Chemicals) and the blood was withdrawn. To the blood was added five volumes of cold methanol, then, these were mixed with 0.3 ml (oily) of nonlabeled synthetic bromine compound as a carrier for extraction of a trace amount of the endogenous bromine compound which may be metabolically labeled with the ^{14}C -activity. The methanol mixture was filtered into a vessel. The filtrate was evaporated under reduced pressure at a low temperature. After most of methanol was depleted by the evaporation, the residue was lyophilized. The lyophilized matter was repeatedly extracted with chloroform. The extracts were combined and condensed to about 1 ml by evaporation under reduced pressure. The condensed sample was supplied for

primary column chromatography, as was done for the third chromatography. The condensed sample which was obtained by this column chromatography was applied to the second thin layer chromatography. The chromatography was carried out as follows. A column packed with Wako Gel C-200 (Wako Pure Chemicals, Tokyo) (12×90 mm) was prepared with chloroform, and then eluted with a mixture of chloroform and carbon tetrachloride (50:50, v/v). Three ml of eluate was fractionated into glass tubes. An aliquot of 0.1 ml from each glass tube was mixed with 3.0 ml of 0.1% methanol solution of ferric chloride. The extinction (E) at 520 nm was pursued to measure the quantity of the bromine compound. The radioactivity (dpm) was measured for an aliquot of 0.1 ml from each glass tubes.

RESULTS AND DISCUSSION

We studied, first of all, a thin layer chromatography of synthetic ^3H - γ -Br (Fig. 1) and then two dimensional thin layer chromatography of synthetic ^3H - γ -Br that was combined with DANSYL L-cysteine (Fig. 2).

In Fig. 1A, three spots were observed on thin layer chromatography of synthetic ^3H - γ -Br, upon spraying with 0.1% FeCl_3 methanol solution. Spot A was violet brown in reaction to the reagent and R_f 0.42, spot B yellowish brown R_f 0.27 and spot C violet brown R_f 0.14.

Spot A was extracted with cold chloroform. Chloroform was evaporated off under the reduced pressure. The residue was re-dissolved in CDCl_3 for NMR examination as shown in Fig. 1B. Each peak corresponded to the chemical residues (BrCH_2^- etc.) illustrated in the spectrogram, suggesting that the spot A substance has the chemical structure of a six-membered ring form of the enol by intramolecular hydrogen bonding between the hydroxyl group and the carbonyl group of the ester function as cyclic γ -Br (Fig. 1C).

Spot B was keto form of 1-methylheptyl

γ -bromoacetoacetate (keto γ -Br) as reported in a previous report (Yanagisawa and Yoshikawa 1973). Most of the freshly synthesized 1-methylheptyl γ -bromoacetoacetate exists in this form. The amounts of spot A substance was 10 to 30% of the spot B substance. Concerning the radioactivity, spot B (2982 dpm) had the greatest radioactivity, followed by spot A (1305 dpm), giving the ratio 30:70 for spot A:spot B.

Spot C, present in only small amounts, had the radioactivity (71 dpm) and gave a positive reaction to the enol test and seems to be enol γ -Br. A part of keto- γ -Br was converted to enol γ -Br by tautomerism, then cyclic γ -Br was produced from the enol form. Back reaction occurs in both steps. Therefore the same hybrid containing these three forms was produced from each of three forms after standing for a period. When the equilibrium was attained, proportion of cyclic γ -Br to keto γ -Br was 1 to 9 or 3 to 7, according to the solvent used. But, these reactions were not so rapid under the low temperature to disturb the performance of this experiment.

As shown in Fig. 1A, three fluorescent components were observed on TLC under ultraviolet light. R_f value of F1, F2 and F3 was 0.51, 0.50 and 0.38, respectively. F1 had a maximum fluorescence emission at 450 nm and excitation maximum at 380 nm and corresponds to di-1-methylheptyl-2, 5-dioxocyclohexane-1, 4-dicarboxyl-ate (DOC) as reported in a previous report (Yanagisawa and Yoshikawa 1973). It seemed that this substance was converted rather rapidly from spot A substance at a warmer temperature, but the true mechanism is obscure because spot A, B and C were spontaneously interconverted each other by tautomerism.

F2 substance had a maximum emission at 445 nm and an excitation maximum at 385 nm. F3 substance had a maximum emission at 440 nm and an excitation maximum at 385 nm. The dioxo-part of the DOC structure can be converted partially to semienol or dienol forms,

Fig. 1. Analysis of synthesized 1-³H-1-methylheptyl γ -bromoacetoacetate A: Thin layer chromatogram of the synthesized 1-³H-1-methylheptyl γ -bromoacetoacetate; B: NMR spectrum of the fraction A of the synthesized 1-³H-1-methylheptyl γ -bromoacetoacetate; C: Possible scheme of the chemical equilibrium among synthesized 1-³H-1-methylheptyl γ -bromoacetoacetate.

Fig. 2. Two dimensional development of the DANSYL L-cysteine combined synthesized 1- 3 H-1-methylheptyl γ -bromoacetoacetate by thin layer chromatography. Spot a and spot b are main spots having the radioactivity and homogeneous. Other spots were artifacts which were derived from the DANSYL reagent.

but this has not been fully ascertained.

Fig. 2 shows the patterns of two dimensional thin layer chromatography of the DANSYL L-cysteine derivatives prepared from 3 H- γ -Br. Each spot was extracted with methanol and supplied for further experiments. Consequently, spot b (1400 dpm) had the greatest radioactivity, followed by spot a (520 dpm) and spot c (31 dpm), giving the ratio of 27:73 for spot a:spot b. The ratio of the radioactivity of spot a to spot b coincided well with the ratio of the radioactivity of spot A to spot B, demonstrating that spot a (DANSYL L-cysteine combined cyclic form of γ -Br) is derived from the spot A, and that spot b (DANSYL L-cysteine combined keto form of γ -Br) is derived from the spot B. At pH 8.0, cyclic γ -Br could not react to form DANSYL-cysteine

Fig. 3. Two dimensional development of the DANSYL L-cysteine combined 1-Methylheptyl γ -bromoacetoacetate in the human blood by thin layer chromatography. Spot a corresponding to cyclic γ -Br was detected, but spot b of keto γ -Br was not observed in blood.

derivative.

Fig. 3 shows the results of two dimensional thin layer chromatography of the bromine compounds in human plasma treated with DANSYL L-cysteine. As a result, spot a that was observed in Fig. 2 was clearly observed, but spot b was not.

When another aliquot of the ether extract of the plasma was left to stand at room temperature for a week and was treated in the same way as above for thin layer chromatography, the chromatographic pattern showing the spot a and the spot b, was similar to that of 3 H- γ -Br in Fig. 2, i.e., the spot b substance appeared in the extract (data not shown). This result indicates that spot b is derived from spot a during long period preservation of spot a substance at room temperature. Therefore, we carried out thin layer chromatography of spot a after stand-

Fig. 4. Rechromatographic analysis of DANSYL L-cysteine combined 1-methylheptyl γ -bromoacetoacetate in the human blood after standing for 8 days, by thin layer chromatography. Lane number 1, 2 and 3 in the figure correspond to the chromatographic pattern of the methanol extract of spot b shown in Fig. 2, spot a shown in Fig. 2, spot a shown in Fig. 3 respectively.

ing of spot a extract for 8 days at room temperature (Fig. 4). In the spot a extract appeared spot b during 8 days at room temperature, confirming that spot b substance was derived from spot a substance, as mentioned above. In the experiment of DANSYL-cystein derivatives of the bromine compound, tautomerism and formation of cyclic γ -Br observed on the free γ -Br were also observed on the DANSYL-cysteine derivatives.

In order to ascertain that spot a in the plasma extract is comparable to spot a for authentic γ -Br in Fig. 2, the fluorescence spectra of these spots were compared (Fig. 5). It was found that the fluorescence spectra of spot a from the plasma extract coincided well spot a for authentic γ -Br, showing that spot a from the plasma extract is a cyclic γ -Br. Quantitative measurement of cyclic γ -Br in the blood plasma was possible by the use of ^3H - γ -Br for isotopic dilution method. For example, 412 p mol/ml in the blood plasma of a person and 546 p mol/ml of another one.

Since butyrate is a possible precursor of γ -Br, we studied the incorporation of ^{14}C -labeled butyrate into γ -Br in the blood of rats. In the experiment using ^{14}C -butyrate, the extract prepared from the rat blood was sup-

plied for the first column chromatography which was carried out by the system described in Materials and Methods. But, keto γ -Br and cyclic γ -Br could not be clearly separated by the chromatography. Eluted fractions showing radioactivity and positive reaction to the reagent of ferric chloride were collected and condensed to a minimum. The condensed matter was applied to the second thin layer chromatography by the system of Fig. 1A. Keto γ -Br (spot B) and cyclic γ -Br (spot A) were extracted separately from the thin layer plate. Both extracts were condensed and supplied for the third column chromatography, as illustrated in Fig. 6. The radioactivity was incorporated selectively into cyclic γ -Br and not into keto γ -Br. Peaks of the radioactivity and extinction at 520 nm of ferric chloride reaction were well consistent. Furthermore, to ascertain whether the ^{14}C is truly incorporated into cyclic γ -Br as a molecular constituent, it was examined whether a fluorescent substance F1 in Fig. 1A was produced spontaneously from two moles of the bromine compound. This reaction is most specific for the bromine compound. Therefore, the radioactivity incorporated into cyclic γ -Br must be recovered also in F1 (DOC). Fractions of cyclic γ -Br shown in Fig. 6 were

Fig. 5. Comparison of fluorescent spectra of spot a obtained from the experiments of ^3H - γ -Br and the human blood.

collected and condensed to a minimum and re-dissolved in a small amount of methanol. The solution was left to stand at room temperature for 18 hours. Then F1 (DOC) was separated from the sample by a thin layer chromatography shown in Fig. 1A. From 3100 dpm of cyclic γ -Br was converted 1414 dpm of F1 (DOC). This experiment demonstrates that the ^{14}C -radioactivity observed in cyclic γ -Br was constituted in the molecule.

Most of the chemically synthesized 1-methylheptyl γ -bromoacetoacetate exists as the keto form. After standing for a period of time, enol form induced from the keto form by tautomerism and cyclic form spontaneously converted from the enol form were detected by thin layer chromatography as shown in Fig. 1. Chromato-graphic treatment involving procedures of extraction and condensation of the

sample seemed to accelerate the tautomerism, when it was treated under room temperature. Therefore all procedures were carried out under low temperature and it could be possible that the tautomerism was reduced to a minimum without interference for further experiments, but it was preferable that chromatographic procedures were completed in a few-hours. For resolution of a confusion caused by presence of three forms, thin layer chromatography of synthetic ^3H - γ -Br (Fig. 1) and two dimensional thin layer chromatography of synthetic ^3H - γ -Br that was combined with DANSYL L-cysteine (Fig. 2), were used.

We consider now that in the chemically synthesized γ -Br is initially produced as the keto-form, which may then be converted to the enol form and further to cyclic form (Fig. 1C).

Fig. 6. Incorporation of ^{14}C -activities into $\gamma\text{-Br}$ in the blood of rat. Open circle in the figure shows the FeCl_3 reactivity of the extracts. Closed triangle in the figure shows the isotopic counts of the extracts. The radioactivities against the extinction at 520 nm were 3630 dpm/E in cyclic $\gamma\text{-Br}$ and 44 dpm/E in keto $\gamma\text{-Br}$.

Interestingly, we found that $\gamma\text{-Br}$ was not present as the keto form but was present as the cyclic form in the human blood (Fig. 3) and in the blood of rats (Fig. 6). However, the mechanism why only the cyclic form of $\gamma\text{-Br}$ was present in the blood is not clear at all, at present, though the chemically synthesized $\gamma\text{-Br}$ is composed of three forms, where the cyclic form is gradually formed from the keto-form via enol-form, through tautomerism. Our speculative view is that there may exist some enzymatic system to promote tautomerism, or to produce cyclic-form in the blood, but should be proved by further studies. When Yanagisawa and Yoshikawa (1967, 1968) found the presence of $\gamma\text{-Br}$ in the CSF, these three forms were not known. Thus, it will be interesting to analyze the form of $\gamma\text{-Br}$ in the CSF. Furthermore, Boger et al. (1998) found that oleamide may play an important role in sleep, as an endogenous sleep-inducing lipid, and that $\gamma\text{-Br}$ is a strong inhibitor of fatty acid amide hydrolase degrading oleamide. Sourkes (1991) stressed the significance of bromide compound as central

nervous system drugs. In the light of these studies, biological effects of the cyclic form of $\gamma\text{-Br}$ awaits further investigation.

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